A STUDY OF THE GASEOUS REQUIREMENTS FOR THE GROWTH OF VARIOUS BACTERIA

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It is generally believed that most bacteria require oxygen for their growth and that the oxygen is obtained directly from the atmosphere, or indirectly through enzyme action from carbohydrates, proteins or other reducible bodies.

That the relation of the growth of bacteria to their gaseous environment might not be quite so simple was suggested by work on the so-called partial tension strains of bacteria; and especially by the observation of Wherry and Ervin [1] that the removal of the CO_2 given off by the bacteria prevented the growth of a recently isolated strain of the tubercle bacillus.

EXPERIMENTS

In a series of experiments during 1919, it was noted that many strains of bacteria—a saprophytic tubercle bacillus, the hay bacillus, staphylococcus aureus, and the gonococcus—would grow very well under anaerobic conditions as provided by displacement with hydrogen, but not at all or only poorly under anaerobic conditions produced by pyrogallic acid and alkali. Later an effort was made to try to explain the discrepancies that resulted from the use of these two methods of producing anaerobic conditions.

Exper. 1.—Aerobic, facultative, partial tension, and anaerobic bacteria were grown under five different gaseous environments, produced as shown in the illustration. Equal inoculations from a suspension were always made.

1. Aerobic sealed. This tube contained O_2 and small quantities of CO_2 given off by the respiration of the bacteria.

2. Aerobic sealed with NaOH solution on the cotton plug. This tube contained O_2 and no CO_2.

3. Under H_2. This tube contained H_2 and small quantities of CO_2 given off by the bacteria.

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[1] Jour. Infect. Dis., 1918, 22, p. 194.

4. Under H_2 plus alkali. This tube contained no O_2 or CO_2.

5. Under pyrogallic acid and alkali. This tube contained no O_2 or CO_2.

The results of this experiment are given in table 1.

TABLE 1

RESULTS OF EXPER. 1

	Aerobic				Facultative				Partial Tension	Anaerobic	
	Hay Bacillus	Tubercle Bacillus 801	Tubercle Bacillus 802	Tubercle Bacillus 803	B. coli	Staphylococcus aureus	Proteus vulgaris	B. anthracis	Gonococcus	B. welchii	B. tetani
Aerobic sealed (O_2 + CO_2)	++ ++	++ ++	++	+	++ ++	++ ++	++ ++	++ ++	+ —	—	—
Aerobic + alkali sealed (O_2, no CO_2)	++ +	++ ++	++	—	++ +	++ +	++ +	++ ++	+	—	—
H_2 (CO_2)	++	++ +	++ +	—	++	++	+ +	++ ++	++ ++	++ +	+ —
H_2 + alkali (No O_2, nor CO_2)	—	—	—	—	+ —	+ —	+ —	+ —	++	++ ++	+
Pyrogalic acid + alkali (No O_2, nor CO_2)	—	—	—	—	+ —	+ —	+ —	+ —	—	++ ++	++ ++

In judging the amount of growth as indicated by + or —, the reading was taken in all instances in 24 hours, except in the case of 801 and 802 (48 hours) and 803 (two weeks). The medium used was beef infusion agar 1% acid to phenolphthalein; 6% glycerol was added to this medium for the growth of the saprophytic tubercle bacilli 801 and 802, while the virulent bovine strain 803 was grown on glycerol egg. Ascites fluid was added to the agar used for the growth of the gonococcus, B. welchii and B. tetani.

In table 1, among the socalled aerobic bacteria, the hay bacillus and the saprophytic tubercle bacilli, and the facultative bacteria, B. coli, Staphylococcus aureus, B. proteus and B. anthracis, all made their maximum growth under sealed aerobic conditions. The growth was somewhat arrested when the respiration CO_2 was removed by adding alkali to the aerobic condition; still less growth occurred under H_2; while under conditions furnished by H_2 and alkali, or by pyrogallic acid and alkali little or no growth occurred. The interesting point in this observation is that the aerobic and facultative bacteria would grow under H_2, yet when alkali was added to the H_2 tube little or no growth

appeared. That is, when alkali was added to the hydrogen tube anaerobic conditions were produced which simulated that produced by pyrogallic acid and alkali. Therefore it seems that the growth of aerobic and facultative bacteria are not only influenced by O_2 but also by CO_2, as they are able to grow under O_2 alone, or $H_2 + CO_2$ alone, but they require one or the other or both of these gases. In other words, in producing anaerobic conditions two factors must be considered: first, oxygen removal, and second, carbon dioxide removal.

Strain 803 of the tubercle bacillus, a pathogenic bovine strain, grew only under sealed aerobic conditions, the removal of CO_2 preventing its growth. It seems therefore that a pathogenic tubercle bacillus requires both O_2 and CO_2 for growth, being unable to grow under O_2 alone or traces of CO_2 alone.

In the case of one partial tension strain tested, the gonococcus, growth occurred under all conditions except the anaerobic as produced by pyrogallic acid and alkali; similar results were reported by Rockwell and McKhann.[2] Under H_2 the gonococcus made a very confluent growth; under H_2 and alkali a number of large colonies appeared, while under pyrogallic acid and alkali no growth occurred. This shows that there is some difference between the three methods of producing anaerobic conditions, namely, displacement by H_2, H_2 and alkali, and pyrogallic acid and alkali.

It is evident that the chemical composition of the medium may decidedly influence the respiration of bacteria. This was clearly shown by the fact that when 1% glucose was added to the nutrient agar B. coli, B. proteus vulgaris and the hay bacillus grew equally well under H_2, H_2 and alkali, and pyrogallic acid and alkali, probably because the evolution of CO_2 exceeded its absorption.

It will be noted that in the case of the anaerobes, B. tetani grew poorly under H_2, slightly better under H_2 and alkali and very well under pyrogallic acid and alkali, again indicating that the three anaerobic methods employed produce different results.

B. welchii gave a better growth under H_2 the first 24 hours than under pyrogallic acid, but in 60-72 hours the growth under the pyrogallic method caught up and passed that under H_2. This last observation was further supported by the following experiments:

Exper. 2.—B. welchii was grown under H_2, $H_2 + 25\%$ CO_2, $H_2 + 50\%$ CO_2, $H_2 + 75\%$ CO_2 and CO_2. The result was that growth

[2] J. Infect. Dis., 1921, 28, p. 249.

occurred in every tube except the ones containing 75% or more of CO_2, that is, a large excess of CO_2 interferes with its growth.

Exper. 3.—An equal inoculation of B. welchi was made into 5 tubes containing 5 c c of litmus milk. These were grown under various gaseous conditions. The gaseous environment and the results of two experiments are shown in table 2.

TABLE 2

RESULTS OF EXPER. 3

	Appearance of Stormy Fermentation in Hours	
	1	2
Aerobic sealed	30	—
Aerobic sealed + alkali	19	20
H_2	16	17
H_2 + alkali	15	16
Pyrogallic acid + alkali	15	16

It is apparent from this experiment that the gaseous environment influenced the period required for growth in milk and fermentation of milk by B. welchii, just the removal of CO_2 by alkali shortening the period considerably.

Judging by these experiments on anaerobes it is probable that CO_2 as well as O_2 influences their growth; further, it is again apparent that the anaerobic condition furnished by H_2, H_2 and alkali, and pyrogallic acid and alkali, are not the same.

SUMMARY AND CONCLUSIONS

Having observed that some aerobic and facultative bacteria would grow under H_2 but not when pyrogallic acid and alkali were used to remove oxygen, further investigations were made. When planted on nutrient agar containing a trace of muscle sugar, the aerobic and facultative bacteria tested, two saprophytic tubercle bacilli, the hay bacillus, B. coli, staphylococcus aureus, proteus and B. anthracis, grew well in sealed aerobic cultures irrespective of whether the CO_2 produced by the bacteria was or was not absorbed by alkali. They also grew well under H_2 when the CO_2 produced by the bacteria was allowed to accumulate, but growth was arrested by placing alkali on the cotton plug of such H_2 cultures; nor did growth appear when pyrogallic acid and alkali were used. In other words, the growth of some bacteria ordinarily considered to be of the aerobic and of the facultative group, is in some way favored by CO_2.

In the presence of glucose, B. coli, B. proteus and the hay bacillus grew equally well under H_2, H_2 and alkali, and pyrogallic acid and alkali, probably because the evolution of CO_2 exceeded its absorption.

In the case of a partial tension strain of the gonococcus, the best growth occurred under H_2 when the exhaled CO_2 was allowed to accumulate and was somewhat diminished by its removal; but no growth appeared in cultures made anaerobic by pyrogallic acid and alkali.

In the case of the anaerobes, B. welchii and B. tetani, the growth of B. welchi was favored by the removal of the exhaled CO_2; B. tetani grew best when the O_2 and CO_2 were removed by pyrogallic acid and alkali. It is not clear just why B. tetani failed to grow well under H_2 unless these cultures still contained small amounts of O_2. If so, this would point to the inability of strict anaerobes to use even traces of atmospheric O_2.

While many points concerning the influence, and the nature of the influence, of the gaseous environment on the growth of bacteria are still obscure, attention to the points brought out in this article may help in the isolation and differentiation of species.

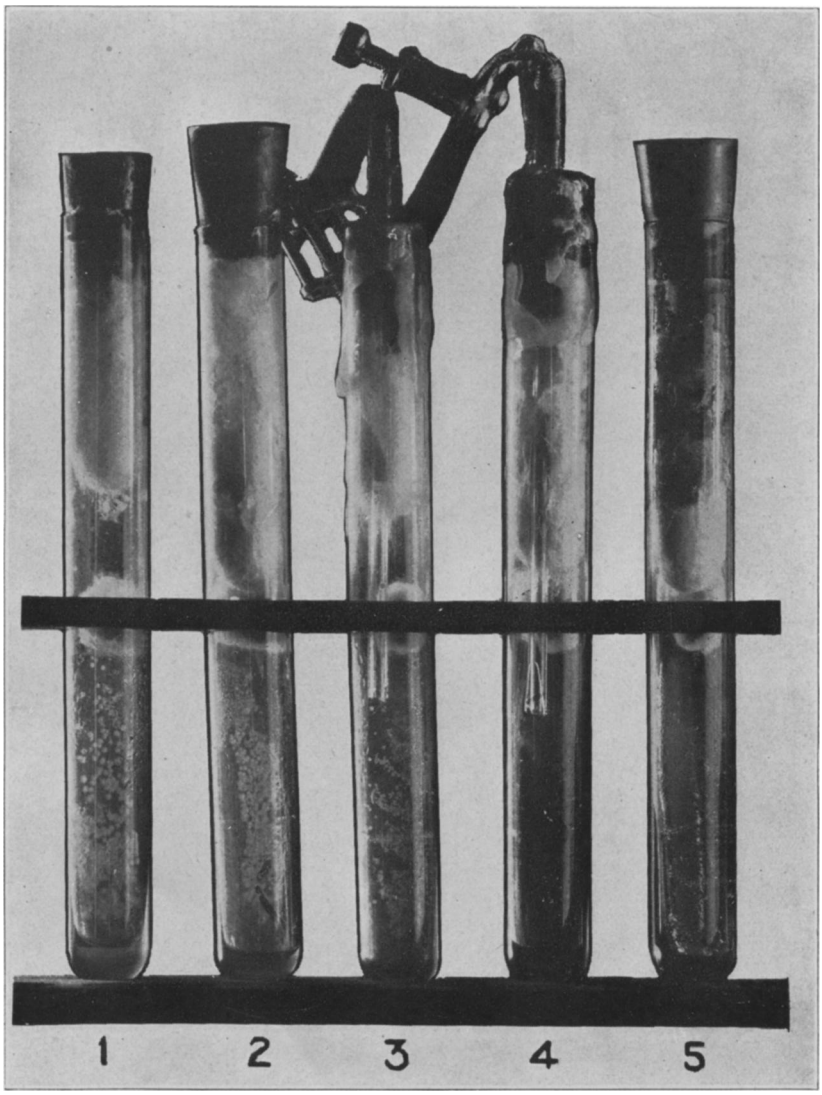

Fig. 1.—Photograph of the growth of B. coli as described in exper. 1. Tube 1 contains aerobic bacteria sealed; tube 2 contains aerobic sealed plus NaOH solution on the cotton plug; tube 3 shows anaerobic conditions produced by displacement with H_2; tube 4 shows anaerobic conditions produced by H_2 plus NaOH solution on a cotton plug; and tube 5 shows the anerobic condition produced by pyrogallic acid and alkali. The method of displacing air with H_2 was the same as used by Rockwell and McKhann.[2]